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14. ABSTRACT

Heart disease is the leading cause of death in both the United States and Hawaii. According to the Hawaii State Department of Health, over 1/3 of total deaths in the state are caused by cardiovascular disease, in which approximately 18% (hospital discharges) were associated with heart failure. The potential of stem cells for use in cell therapy to treat diseased or damaged organs is very promising due to the unique properties of these cells, namely the capacity for both long-term self-renewal and differentiation into various mature cell types. There are 2 main objectives for our research, 1) to examine the efficiency of repair and recovery of damaged heart tissue in stem cell based therapies by using enriiched hematopoietic stem cells (HSC) and 2) study the efficiency of trans-differentiation of HSC into cardiomyocytes, following transplantation into damaged (ischemic) heart tissue to promote cardiogenesis.

15. SUBJECT TERMS
Hematopoietic stem cells, congestive heart failure, stem cell therapy

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Introduction

Heart disease is the leading cause of death in both the United States and Hawaii. According to the Hawaii State Department of Health, over 1/3 of total deaths in the state are caused by cardiovascular disease, in which approximately 18% (hospital discharges) were associated with heart failure (Balabis, Pobustsky, Kromer Baker, Tottori, & Salvail, 2007). Many patients with end-stage congestive heart failure (ESCHF) reach a state where medical therapy is not adequate to sustain acceptable cardiac function. After medical therapies have been optimized, further therapeutic options are limited to mechanical circulatory support (MSC) systems such as Ventricular Assist Devices (VADs), cardiac transplantation, or highly experimental stem cell procedures; none of these options are available in Hawaii.

The potential of stem cells for use in cell therapy to treat diseased or damaged organs is very promising due to the unique properties of these cells, namely the capacity for both long-term self-renewal and differentiation into various mature cell types. While it may be ideal to treat different organs or tissues with stem cells of the same type (i.e., treat brain with brain-derived stem cells), in many instances tissue- or organ-specific stem cells have either not yet been identified, or the isolation of these stem cells is impractical. Hematopoietic stem cells (HSC) are presently one of the most attractive types of stem cells for use in cell therapy because they are well characterized, are relatively easily purified, and can be isolated directly from either mobilized blood in adults or from cord blood. In particular, bone marrow HSC are of interest because they may be used in autologous transplants.

In this study, we propose to further examine the potential and mechanism of HSC to treat damaged or diseased heart tissue, by (1) comparing the capacity of highly purified human HSC samples, bone marrow progenitor/stem samples, and whole bone marrow samples to promote the recovery of heart tissue in mice following transplantation to treat myocardial infarct, and (2) examine whether HSC can be re-programmed towards cardiogenesis, instead of hematopoiesis, by treating these cells with specific growth factors and/or expressing genetic regulators of cardiopoiesis in these cells.

If this proves possible, then the ability of these cells to promote the recovery of heart tissue in mice following transplantation to treat myocardial infarct will be compared to that of untreated HSC. These results should shed light on how important HSC are with respect to use in cell therapy to treat myocardial infarcts, and may also lead to an improved cell therapy based method, involving the re-programming of donor cells to a cardiogenic fate prior to transplantation, to treat damaged or diseased heart tissue.

Body

The following are a summary of activities during this period.

1. Complete all appropriate procedures with institutional review boards - ***completed***
    a. Local University of Hawaii Animal Care & Use committee has approved this protocol on 15 January 2009.
        i. Local University of Hawaii Animal Care & Use committee has approved Amendment #1 for this protocol on 16 July 2009.
            1. An alternative way to induce myocardial infarct in the mice has been identified and included as an alternative optional method to this protocol.
            2. Small change in protocol to utilize new end-point measures of angiogenesis, since the field is constantly changing and evolving, and we want use the best measure as we can.
    b. The USAMRMC Animal Care and Use Review Office has approved this protocol on 21 April 2009.
        i. The USAMRMC Animal Care and Use Review Office has approved Amendment #1 of this protocol on 18 August 2009.
    c. Local University of Hawaii Committee on Human Studies has approved this protocol as an exempt study, under DHHS regulation, 45, CRF Part 46 on 21 January 2009.
    d. The USAMRMC Office of Research Protections, Human Research Protection Office has reviewed this protocol and, in accordance with 32 CFR 219.102(f), the HRPO determined that the proposal constitutes research not involving human subjects. Determination was received on 21 April 2009.
2. Conduct stem cell research for the treatment of congestive heart failure. Studies will examine the potential and mechanism of hematopoietic stem cells to treat damaged or diseased heart tissue
    a. Hypothesis: Are hematopoietic stem cells (HSC) required to promote recovery in cell-based therapies that utilize bone marrow derived samples to treat myocardial infarct?

        The training of Dr. Allsopp's lab members in both the initial protocol and alternative protocol to induce myocardial infarct is complete. 40 C57BL6 mice were used to develop the model.

        We received our first bone marrow sample from Hawaii Transplant Center in early December, however, the sample was not large, and later determined to be of poor quality (excessive clotting). In early January, we were able to acquire a second larger bone marrow sample, which is of good quality. This sample should be sufficient for all experiments proposed in this study. We have initiated optimizing our stem cell sort protocol with these samples, which is near completion. An order for 40 SCID

mice has been placed for the initial transplant experiments, which we plan to begin in early March 2010.

b. Hypothesis: Will exposure of HSC to specific re-programming factors promote the ability of HSC to trans-differentiate into cardiomyocytes following transplantation into damaged heart tissue?

   We have completed the cloning of the human Nkx2.5 cDNA, and are presently constructing the lentiviral expression vector to use in experiments to re-program HSC into cardiac progenitor cells in vitro. We have now completed assembly of the retroviral Nkx2.5 expression vector, and have confirmed Nkx2.5 expression in human cell lines in vitro. Frozen aliquots of the packaged lentivirus have been prepared and stored for use as soon as donor bone marrow samples have been obtained.

   See update above for first hypothesis (a) in regards to acquiring bone marrow samples. We are close to completing optimization of sort conditions for isolating bone marrow (hematopoietic) stem cells from the bone marrow samples, and plan to begin experiments using the lentivirus and sorted stem cells in early March 2010.

3. Analyze data, interpret results, and draft manuscript for publication – this task will be completed upon completion of the stem cell research. Without animal entry at this time, there is no data to review.

Key Research Accomplishments

1) **Task 1. Complete all appropriate procedures with institutional review boards**
   a) Protocols for this research has been submitted and approved by the following organizations.
      i) University of Hawaii Animal Care & Use
      ii) University of Hawaii Committee on Human Studies
      iii) USAMRMC Animal Care and Use Review Office
      iv) USAMRMC Office of Research Protections, Human Research Protection Office
2) **Task 2. Conduct stem cell research for the treatment of congestive heart failure. Studies will examine the potential and mechanism of hematopoietic stem cells to treat damaged or diseased heart tissue**
      i) Hypothesis: Are hematopoietic stem cells (HSC) required to promote recovery in cell-based therapies that utilize bone marrow derived samples to treat myocardial infarct?
         (1) Training of Dr. Allsopp's lab members in both the initial protocol and alternative protocol to induce myocardial infarct is complete. 40 C57BL6 mice were used to develop the model.
         (2) We received bone marrow samples from Hawaii Transplant Center and have initiated optimizing our stem cell sort protocol with these samples, which is near completion.
         (3) An order for 40 SCID mice has been placed for the initial transplant experiments, which we plan to begin in early March 2010.
      ii) Hypothesis: Will exposure of HSC to specific re-programming factors promote the ability of HSC to trans-differentiate into cardiomyocytes following transplantation into damaged heart tissue?
         (1) We have completed the cloning of the human Nkx2.5 cDNA, and are presently constructing the lentiviral expression vector to use in experiments to re-program HSC into cardiac progenitor cells in vitro.
         (2) We have now completed assembly of the retroviral Nkx2.5 expression vector, and have confirmed Nkx2.5 expression in human cell lines in vitro.
         (3) Frozen aliquots of the packaged lentivirus have been prepared and stored for use.
         (4) We are close to completing optimization of sort conditions for isolating bone marrow (hematopoietic) stem cells from the bone marrow samples, and plan to begin experiments using the lentivirus and sorted stem cells in early March 2010.

Conclusions

In accordance with the newly established Armed Forces Institute of Regenerative Medicine (AFIRM), our research aims to find innovative ways to treat heart disease. Regenerative medicine offers hope for better treatment and cures for chronic diseases, which will improve the lives of veterans and citizens. As proof, bone marrow transplantation is a well-established therapy for a variety of blood diseases; however, other applications using adult stem cells are still largely in the research phases. In order to demonstrate the safety and efficacy, extensive research is needed. Results of this research will benefit all citizens, including military retirees, recuperating from congestive heart failure.

Heart failure and heart disease are one of the most common afflictions of the elderly and are also one of the most common causes of both morbidity and mortality (Cosentino & Osto, 2007). Therefore, the development of new methodologies to treat and improve prognosis of cardiac patients is highly desired. One of the key points of significance of this research is that the results could ultimately lead to the development of a novel and improved method to treat heart disease and ischemic damage. Relative to other stem cell-based therapies that have been proposed, for example procedures which rely on embryonic stem cells or cardiac stem cells, our procedure has the advantage of using stem cells that are readily available from the patients own bone marrow (Strauer, Brehm, & Schannwell, 2008). In addition, autologous transplantation of the patients' own HSC would eliminate the risk of rejection of the transplanted cells by the host immune system.

The results from this research are expected to ultimately lead to improved methods for stem cell-based therapies to treat damaged (ischemic) heart tissue. Specifically, it is expected that if the modifications, as described in this proposal, to stem cell (HSC) based therapy for treating myocardial infarcts can improve recovery from myocardial infarcts in lab animals, then these findings will also translate to stem cell therapy used to treat human cardiac patients.

References

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